

REMARKS

Claims 1-7, 14-18, 38-50, 60-61, 65, 67, and 68 are pending; claims 8-13, 19-37, 51-59, 62-64, and 66 having been cancelled by the above amendment. Claims 15 and 17 are withdrawn; however, the Applicant earlier requested rejoinder pursuant to MPEP § 806.04(d) upon a finding that claim 1 is allowable.

Telephonic Interview of 17 May 2005

The Applicant thanks Examiner Moran for the courtesy of the telephonic interview conducted 17 May 2005. To briefly summarize, the Applicant clarified that the method of claim 1 is a method of protein design, rather than a method of predicting the side chain conformation of a known sequence. Examiner Moran expressed initial agreement that Koehl 1994¹ does not teach protein design, although the Applicant appreciates that the Examiner will verify this conclusion. The Applicant also explained that neither Koehl 1994 nor Dahiyat² teaches an ensemble of related backbone structures. Examiner Moran further explained the § 112 rejections in the office action and offered suggestions for possible amendments. The Applicant's position on these and other issues is further elaborated below.

Rejection of Claim 1 under § 112 ¶ 2 and Support for "Energetic Fitness"

The Examiner alleged that:

Amended claim 1 recites a step of sampling or evaluating "fitness" of one or more amino acids. Claim 19 recites a step of "evaluating fitness" of amino acids. Claim 56 also recites "evaluating fitness" of rotamers. New claim 67 recites "evaluating fitness" of amino acids by "evaluating fitness" of rotamers. It is unclear what "fitness" is being evaluated in these claims. The term "fitness" is not defined by the specification, and it is unclear whether "fitness" is intended to be a qualitative or quantitative evaluation. For example, is "fitness" associated with energy constraints, torsional constraints, some other physical parameter, fitting or docking to a binding site on another protein or

¹ Koehl et al. (1994) *J. Mol. Biol.* 239:249-275.

² Dahiyat et al. (1996) *Protein Sci.* vol. 5, pp. 895-903.

receptor, or some combination of parameters? As it is unclear what "fitness" is to be evaluated, the claims are indefinite.

Without conceding the point, claim 1 has been amended to refer to "energetic fitness." The specification is replete with references to the use of energy as a measure of fitness. For example, the application refers to:

Location	Text
12:28-29	"sampling of side chain identities and orientations in a combinatorial search for low <u>energy</u> structures"
18:14-16	"use of defined field probabilities or free <u>energy</u> values that represent the viable amino acid sequence space for a protein fold,"
16:14	"the total <u>energy</u> of each sample state"
17:13-29	a partition function that includes, as a variable, E (the total calculated energy)

Citations above refer to the page:line of the specification as filed.

Each of these exemplary measures of "energy" illustrates the general concept of "energetic fitness" as a gauge of whether an amino acid is fit to occupy a particular position in the polypeptide chain. Examiner Moran expressly indicated during the telephonic conference that the term "energetic fitness" would render the claims definite. With the current amendment, the Applicant submits that the rejection can be withdrawn.

Rejection of Claims 39 & 40 and Support for Amendment to Claims 39 and 40

The Examiner maintains that:

Original claims 39-40 limited a library to be "designed by" various procedures. It was unclear what structural limitation of the library elements was intended by the "design" limitations or if further method steps were intended, as previously set forth. Original claims 57 and 58 (currently withdrawn) recite identifying side chains suitable for a protein structure, but do not recite identification of an entire library of proteins from a probability matrix. The originally filed specification discloses and exemplifies designing and generating proteins and combinatorial libraries; e.g. on pages 26-33, but does not disclose identification of a library of proteins anywhere. Applicant has not set forth support for the newly added limitations of claims 39-40 in the response filed 11/26/04, and none is apparent, as set forth above, therefore the claims are rejected for reciting new matter.

Without conceding the point, the Applicant has amended claims 39 and 40 to include the language ("generating proteins and combinatorial libraries") explicitly recommended by the Examiner and underscored in the passage above. Additional support for the amendment and for "synthesizing" can be found, e.g., page 7, lines 9-12 and page 6, lines 22-25 of the specification.

Rejection of Claim 48 under § 112 ¶ 2 & Amendment of Claim 48

Claim 48 is amended indicate that the library is screened or selected "for a desired property." Support for the amendment can be found, e.g., at page 29, line 28, which refers to "desired properties." The Examiner stated:

Claim 48 limits the method of claim 3 to further comprise screening or selecting one or more proteins from the generated combinatorial library. However, claim 48 does not recite any parameters for screening or selecting, such that one skilled in the art would be apprised of the metes and bounds intended by applicant for the protein to be thus chosen. As is it unclear what the protein is intended to be selected or screened for (or against), the claim is indefinite.

During the telephonic discussion, the Examiner recommended amending the claim to refer to "a desired property." The Applicant has adopted the Examiner's suggestion without conceding the propriety of the rejection.

Rejection of Claim 50 & Support for Amendment to Claim 50

The Examiner maintains that:

The terms "enhanced" and "improved" in claim 50 are relative terms which renders the claim indefinite. The term terms "enhanced" and "improved" are not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. In addition, it is still not clear what is intended by an "enhanced" activity (e.g. a higher binding affinity may be considered "enhanced" for an compound which acts as a receptor agonist whereas a lower binding affinity would be considered "enhanced" for an antagonist). For these reasons and those previously set forth, the examiner maintains that the claim is indefinite.

Without conceding the point, the Applicant has amended claim 50 to refer to "enhanced catalytic activity" and has deleted the reference to "improved." Thus, one skilled in the art

would know what is intended by "enhanced." Support for the amendment can be found, e.g., at page 28, line 29.

Rejection of Claim 68

Claim 68 was rejected for an improper antecedent. The claim has been amended to properly refer back to claim 2, as the Examiner suggested.

Rejection of claim 1 under § 102 in view of Koehl 1994

The Examiner maintains:

KOEHL teaches a computerized method of generating a global conformational (probability) matrix representing a protein structure (p. 250) wherein an averaged rotamer (backbone) library or ensemble is provided (p. 251) a self consistent mean field theory/algorithm (SCFM) is used to generate possible side chain sequences and to evaluate all possible rotamers in "the context" of the backbones and side chain sequences to generate the matrix (pp. 251-252 and 256-257), thus anticipating claims 1, 5, 19 and 44, and 65. KOEHL teaches that the protein and/or backbones may be derived from or based on comparison to a natural protein (pp. 254-255), thus anticipating claims 6-7, 14, 20-21, and 27-28. KOEHL further teaches that her matrix calculations comprise information from partition functions (p. 254) and comprise information for all amino acids (p. 259, esp. Table 3), thereby anticipating claims 45 and 47. KOEHL teaches that his method steps may be iterated in multiple cycles, using multiple matrices, until convergence is reached (e. g.; p. 254), and teaches addition and subtraction of free energy to meet accuracy constraints (pp. 254-258), thus anticipating claims 4, 8, 12-13, 18, 29, 41-43, 46, 49, and 51-56.

However, the method of claim 1 differs substantially from Koehl. These differences include at least two limitations of claims 1 and 65. The first is the application of a protein design algorithm, and the second is the provision of an ensemble of related backbone structures.

1. Protein design differs substantially from predicting the structure of a given primary sequence. A protein design algorithm generates amino acid sequences (typically new amino acid sequences) that satisfy a particular characteristic, e.g., ability to form a particular three-dimensional fold. In a method of protein design, the amino acid sequence is the **unknown**. The input for the method is often a desired three-dimensional fold; the output is one or more amino acid sequences that are predicted to adopt the desired fold.

In contrast, structure prediction relies on a **known** amino acid sequence as its input and produces, as its output, a prediction of side chain conformations for the amino acid sequence. Structure prediction does not generate an amino acid sequence, quite the contrary, an amino acid sequence is the prerequisite for structure prediction.

Whereas the method of claim 1 includes a protein design algorithm, the cited Koehl 1994 reference is emphatically a method of structure prediction that depends on a known amino acid sequence as a prerequisite. Koehl 1994's method merely predicts what side chain conformations might be occupied when the known amino acid sequence is modeled on its known fold. Several passages in Koehl 1994 confirm that Koehl 1994 is restricted to protein structure prediction and has no disclosed connection to protein design:

On page 249, column 2:

In this paper, we shall focus on the side-chain problem, which is a subset of the inverse folding problem.

On page 250:

The basic idea of this method is to attach to each Calpha multiple copies of the same side chain, corresponding to all possible rotamers but with different probabilities. It is related to the procedure proposed by Roitberg & Elber (1991) . . . and simulated annealing to provide side-chain placement. A global conformational matrix CM is built, such that CM(i,j) represents the probability of the side chain of residue i in the protein sequence adopting the conformation described by rotamer j. Each residue is considered in turn; the matrix row corresponding to a residue i is updated, based on the mean field generated by the multiple side-chains at neighbouring residues, and the procedure is repeated till convergence is reached. Thus, this method is a self-consistent mean-field theory. In a sense, each side-chain "feels" an average of all possible conformations of its neighbours and is therefore constrained in a "tube" to use a terminology of polymer physics. The final matrix provides the probabilities of all possible positions for all sidechains of the protein, from which an estimate of the conformation entropy of the side-chains is calculated. The predicted conformation of the side-chain of a residue i is chosen to be the rotamer of i with the highest probability.

The essential elements in this new method are that the computational effort only grows linearly (instead of exponentially) with the number of degrees of freedom, the procedure is fully automated, and an estimate of the full

conformational space available to the side-chains of the protein studied is directly derived. [emphasis added]

Note in the above paragraph, the method never ventures beyond the amino acids of “the protein” or the “protein sequence.” Koehl’s primary example is a prediction of the side chain conformation for rhizopuspepsin, described on pages 254-255. Thus, in this implementation, “the protein sequence” is the amino acid sequence of rhizopuspepsin. Koehl confirms this reading at page 254, column 2, bottom:

As an example, we give detailed information on the convergence of the method ALL for a medium size protein, rhizopuspepsin. It contains 325 residues, and its structure has been solved The side chain optimization was carried over 20 cycles. The initial conformational matrix was set according to equation (12), i.e., all rotamers of a given residue were given the same initial probabilities. . . . In the model structure obtained from the final conformational matrix, 81% of all χ_1 dihedrals and 73% of χ_1 -2 dihedrals were predicted correctly . . . and the global r.m.s.d. over the sidechains was 1.45 Å. [emphasis added]

Moreover, Koehl 1994 never suggests generating an amino acid sequence that can adopt the rhizopuspepsin fold. All Koehl 1994 is concerned with is how the known amino acids of rhizopuspepsin are oriented in the folded structure of the known amino acid sequence. Thus, the method in Koehl 1994 is a method of side chain prediction, not a method of protein design.

Because Koehl does not disclose the use of a protein design algorithm, it cannot anticipate the method of claim 1. Nor can it anticipate the method of claim 65. If the Examiner reaches a contrary conclusion, the Applicant would be grateful if the Examiner would specifically point to the language in Koehl relied upon.

2. Koehl 1994 does not disclose an ensemble of proteins. The Examiner points to page 251 of Koehl 1994:

KOEHL teaches a computerized method of generating a global conformational (probability) matrix representing a protein structure (p. 250) wherein an averaged rotamer (backbone) library or ensemble is provided (p. 251).

In the telephonic discussion, the Examiner indicated that she was referring to the “averaged rotamer library” on page 251. Here, Koehl 1994 states:

The averaged rotamer library of Tuffery et al. (1991) . . . is used with the following modifications. Three additional rotamers were added for proline Three rotamers for Tyr and two rotamers for Glu were removed since they appeared twice The total number of rotamers is 108 for non-glycine and non-alanine amino acids.

As the above passage makes clear, the “averaged rotamer library” used by Koehl 1994 is a list of 108 rotamers. While each rotamer might be defined by bond angles, the list of rotamers does not amount to a backbone structure, much less an ensemble of backbone structures.

To analogize, the rotamer library is like a codon library that provides a list of possible codons for each amino acid. One synthesizing a particular gene might look up an amino acid in the codon library and select a possible codon for incorporation. Similarly, here, when predicting the protein structure of a given amino acid sequence, one might look up possible rotamers of an amino acid that is specified at a particular position from the rotamer library to predict what side chain conformation the amino acid will have in the folded structure. Just as the codon library does not provide a particular gene sequence, the rotamer library does not provide a particular backbone structure.

The absence of rotamers for alanine and glycine further compels this conclusion. These two amino acids either have a very short side chain (a methyl in the case of alanine) or no side chain (glycine). Specifying different side chain orientations for these two amino acids would be meaningless. Accordingly, for these two special cases, there is no information to include in the rotamer library.

To conclude, the Examiner's basis for an “ensemble of related backbone structures” in Koehl 1994 rested on the disclosure of a rotamer library on page 251. This rotamer library, however, only includes information about possible orientations for individual amino acids, not information about a backbone structure, and certainly not information that resembles an ensemble of related backbone structures.

Koehl 1994 did apply its structure prediction method to 30 different test proteins. See, e.g., page 250, column 2, at the bottom:

Full side-chain predictions for 30 proteins from the known backbone are presented: those proteins that have already been used to test other prediction methods were chosen for that purpose. [emphasis added]

However, Koehl predicted the structure (in particular the side chain conformations) for each protein **individually** from its "known backbone." Table 2 on page 258 lists the structures of the 30 test proteins and the RMSD values for the individual predictions. The table makes clear that each structure represents an independent prediction of the sidechain structure for a single protein backbone. The PDB codes in Table 2 also indicate that a diverse set of structures were independently tested. The descriptors of the first five are:

1CPV	Calcium-Binding Parvalbumin B
1CRN	Crambin
1CTF	L7(Slash)L12 50 S Ribosomal Protein (C-Terminal Domain)
1FDX	Ferredoxin
1LZ1	Lysozyme

There is no structural relationship between these different proteins. Rather, the list is a diverse set, as is appropriate for testing the effectiveness of a structure prediction method.

Thus, Koehl 1994 used **unrelated** backbone structures and used each one of them **individually** rather than as an ensemble. Clearly, Koehl 1994 does not teach or suggest an "ensemble of related backbone structures," as required by the methods of claim 1 and 65. Accordingly, Koehl 1994 cannot anticipate these claims.

The Applicant respectfully submits that the anticipation rejection can be withdrawn since Koehl 1994 does not teach every element of claim 1, claim 65 and claims dependent therefrom.

Obviousness Rejection citing Koehl 1994 in view of Koehl 1996

The Examiner further maintains that:

Claims 1,4-8, 12-14, 16, 18-22,24,27-29,41-47,49,51-56 and 64-65 are rejected under 35 USC. 103(a) as being unpatentable over KOEHL et al. (J. Molec. Biol. (1994) vol. 239, pp. 249-275)in view of KOEHL et al. (Current Opinion Struct. Bio. (1996) vol. 6, pp. 222-226).

KOEHL (1994) teaches a computerized method of generating a global conformational (probability) matrix representing a protein structure, as set forth

above. KOEHL does not teach a Monte Carlo algorithm to generate an ensemble of proteins.

KOEHL (1996) teaches a mean field Monte Carlo procedure to generate a family (ensemble) of proteins, and teaches that this provides significant improvement in an SCMF method of modeling proteins (p. 224).

The rejection, as drafted, appears to merely rely on Koehl 1996 to supply an element that the Examiner believed was missing from Koehl 1994 – “a Monte Carlo algorithm.” There is no indication that the Examiner contends that Koehl 1996 teaches the elements that the Applicant has explained are missing from Koehl 1994: for example, use of an “ensemble of related backbone structures.”

Nevertheless, to expedite prosecution, the Applicant notes that Koehl 1996, like Koehl 1994, fails to teach or suggest using an “ensemble of related backbone structures.” In fact, when referring to particular implementations, Koehl 1996 regularly refers to “a protein” and “the protein.” See, e.g., page 222, first paragraph (“for a 50-residue protein”; page 222, last paragraph (“partition of a protein”); page 223, last paragraph (“conformations for a protein with a known backbone scaffold”); page 224, second paragraph (“folded state of the protein”); and so on. Clearly, Koehl 1996 does not teach or suggest an “ensemble of related backbone structures,” as required by the methods of claim 1 and 65. Because neither reference teaches this element, the alleged combination of Koehl 1994 and Koehl 1996 is insufficient to render these claims obvious.

Obviousness Rejection citing Koehl 1994, Koehl 1996, and Dahiyat

The Examiner states:

Claims 2-3,25-26,38-40,48,50, and 60-61 are rejected under 35 U. S. C. 103(a) as being unpatentable over KOEHL et al. (J. Molec. Biol. (1994) vol. 239, pp. 249-275) in view of KOEHL et al. (Current Opinion Struct. Bio. (1996) vol. 6, pp. 222-226) as applied to claims 1,4-8, 12-14, 16, 18-22,24,27-29,41-43,46-47,49,51-56 and 64-65 above, and further in view of DAHIYAT et al. (Protein Sci. (1996) vol. 5, pp. 895-903).

KOEHL and KOEHL et al. teach and make obvious a computerized method of generating a global conformational (probability) matrix representing a protein structure, as set forth above. Koehl (1994) further teaches that her method may be used to predict target structures based on her energy calculations (p. 254).

Neither KOEHL teaches generation or selection of a protein or proteins generated/designed by the method.

DAHIYAT teaches a method of designing proteins from a backbone and rotamer library using a Monte Carlo algorithm (p. 901) and teaches selection and synthesis of the peptide library designed (p. 902). DAHIYAT teaches that proteins may be selected for stability (p. 895: Abstract).

The Applicant understands the underscored language above to incorporate by reference the Examiner's rejection under § 102 in view of Koehl (1994). The Applicant has traversed this rejection above. There is no suggestion from the record that the Examiner is relying on Dahiyat for the use of an "ensemble of related backbone structures."

In any event, Dahiyat does not disclose using an "ensemble of related backbone structures." Dahiyat uses a single backbone structure. See, e.g., page 896, column 1:

The PDA side-chain selection algorithm requires as input a backbone structure defining the desired fold. The task of designing a sequence that takes this fold can be viewed as finding an optimal arrangement of amino acid side chains relative to the given backbone.

Also on page 896, column 2, where the actual computational steps are performed, Dahiyat notes:

We use an extension of the Dead-End Elimination (DEE) theorem to solve the combinatorial search problem. The DEE theorem is the basis for a very fast discrete search algorithm that was designed to pack protein side chains on a fixed backbone with a known sequence.

There is no indication that the DEE theorem can or should be used with an "ensemble of related backbone structures."

Because none of Dahiyat, Koehl 1994, and Koehl 1996 discloses an "ensemble of related backbone structures," the combination of these references cannot make obvious the methods of claims 1 and 65 nor claims dependent therefrom.

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Conclusion

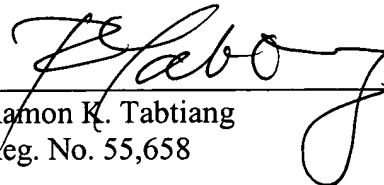
The Applicant respectfully submits that all claims are in condition for allowance, which action is expeditiously requested. The Applicant does not concede any positions of the Examiner that are not expressly addressed above, nor does the Applicant concede that there are not other good reasons for patentability of the presented claims or other claims. All amendments and cancellations are made without prejudice and disclaimer and may be made for reasons not explicitly stated or for reasons in addition to ones stated.

If a telephonic discussion would expedite the examination or prosecution of this application, the Examiner is urged to call the undersigned at 617-521-7017.

Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: 23 May 2005



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